JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1983, p. 1183–1189 Vol. 18, No. 5
0095-1137/83/111183-07$02.00/0

Monoclonal Antibodies Specific for O-Antigenic Polysaccharides of *Shigella flexneri*: Clones Binding to II, II:3,4, and 7,8 Epitopes

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Received 19 April 1983/Accepted 22 August 1983

Hybrid cells producing monoclonal antibodies against the O-antigens of *Shigella flexneri* were obtained by polyethylene glycol-mediated fusion of myeloma cells and lymphocytes from BALB/c mice immunized with whole heat-killed *S. flexneri* bacteria of serotypes 2a and 2b. Clones were selected for their binding specificity to structurally defined *S. flexneri* lipopolysaccharides (LPS). The following three groups were identified as recognizing three different epitopes: monoclonal antibodies binding to (i) *S. flexneri* LPS with the II:3,4 antigens, (ii) *S. flexneri* LPS with the II:3,4 antigens and the II:7,8 antigens, and (iii) *S. flexneri* LPS with the 7,8 group antigen only. Of cloned and characterized antibodies, more than 90% had either the μ or γ_3 heavy chain and 98% had the κ light chain. The exquisite specificity of each monoclonal antibody preparation was in complete contrast to the polyclonal specificities seen in sera from immunized rabbits. Even absorbed rabbit *S. flexneri* typing sera contained antibodies reacting with several different LPS, i.e., they were not type antigen specific. Ascites from immunoglobulin G monoclonal antibody preparations representing the three different specificities were used for sensitizing *Staphylococcus aureus* Cowan 1 bacteria and were used in coagglutination. In testing 211 clinical isolates of all different serotypes of *S. flexneri*, the reagents were shown to be sensitive and specific in correctly identifying all *S. flexneri* II and 7,8 antigen-containing strains with no false positives. Two isolated immunoglobulin M antibody clones specific for the II:3,4 and 7,8 antigens were used as successfully for identification by direct slide agglutination. These results suggest that the monoclonal reagents are superior to conventional typing antisera.

Shigella flexneri bacteria are subdivided into serotypes based on the combination(s) of antigenic determinants present in the O polysaccharide (PS) chains of the cell envelope lipopolysaccharide (LPS) (7). Each serotype usually has one type-specific (I, II, III, etc.) and one group-specific (3,4; 7,8, or 6) antigenic determinant. Antisera for diagnostic purposes are produced by immunization of rabbits, followed by repeated absorptions to render the antibody preparations specific. However, despite much effort, the specificity of the resulting antibody preparations has been questionable. Structural analyses of the O-antigenic PS chains of *S. flexneri* performed in recent years have established that all serotypes (from 1 to 5) have a basic O side chain constructed as shown in Fig. 1, a structure identical to serotype Y. The various serotype specificities are a result of additions of D-glucosyl or acetyl residues or both to the various L-rhamnosyl or the *N*-acetyl-D-glucosaminyl residues of the basic Y structure (Fig. 1) (15–17).

The establishment of the hybridoma technique by Köhler and Milstein (18) has enabled the production of monoclonal antibody preparations with defined specificity. In this paper we describe the production, selection, and characterization of monoclonal antibodies with specificity for three antigenic determinants of *S. flexneri* (MASF). These monoclonal antibodies were shown to be superior to conventional rabbit antisera in terms of specificity and enabled the absolute identification of *S. flexneri* serotype 2 and *S. flexneri* serotypes carrying the 7,8 group antigen.

MATERIALS AND METHODS

Bacterial strains. *Shigella sonnei* and *S. flexneri* strains of serotypes 1a, 1b, 2a, 2b, 3a, 4a, 4b, 5a, 5b, X, and 6 were obtained from the strain collection of the National Bacteriological Laboratory (NBL), Stockholm, Sweden. *S. flexneri* strain F3 (serotype Y) and the bacteriophage Sf 6 lysogenic variant (serotype 3b) were obtained from P. Gemski, Jr., Walter Reed

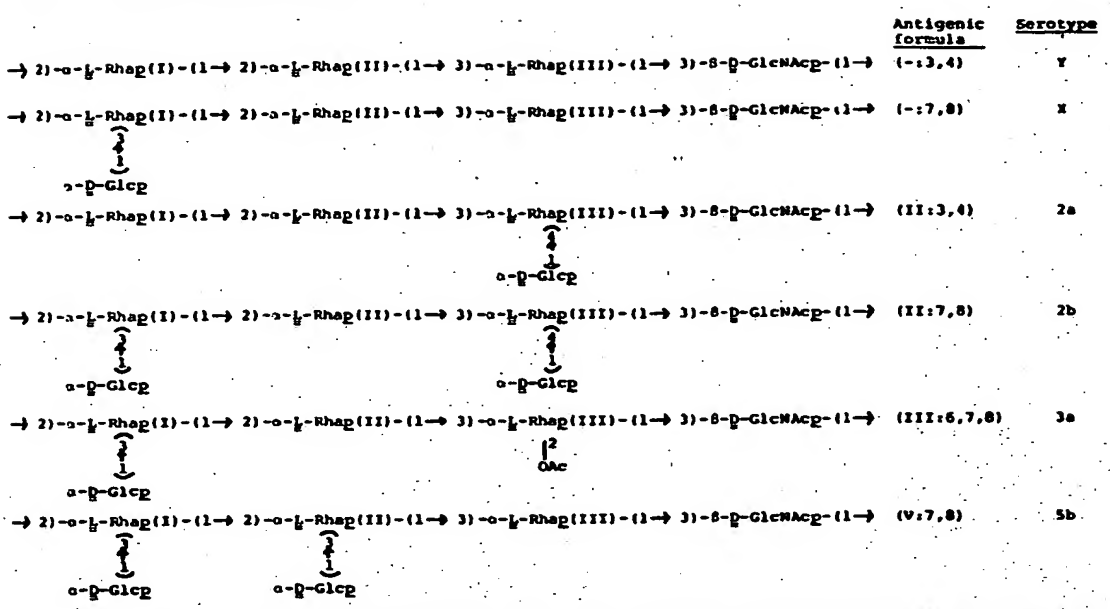

FIG. 1. Structure of the O-antigenic polysaccharide chain in *S. flexneri* LPS. Abbreviations: Rha, rhamnose; GlcNAc, *N*-acetyl-glucosamine; Glc, glucose; OAc, O-acetyl.

Army Institute of Research, Washington, D.C. *Staphylococcus aureus* Cowan 1 was obtained from T. Holme, Department of Bacteriology, Karolinska Institute, Stockholm, Sweden. For coagglutination tests, *S. flexneri* strains from NBL as well as clinical isolates were used.

Preparation of LPS. Bacteria were grown in submerged culture, and LPS were extracted by the phenol-water method from formaldehyde-killed bacteria (27).

Chemical characterization of LPS. Sugar analysis of LPS was performed essentially as described by Sawardeker et al. (25). After hydrolysis and subsequent work-up, the monosaccharide alditol acetates were analyzed by gas-liquid chromatography, using a Perkin-Elmer 990 instrument fitted with a 3% OV-225 column. For nuclear magnetic resonance (NMR) spectroscopy, O-antigenic PS devoid of lipid A were used. Lipid A was cleaved by weak acid hydrolysis (6), lipid was spun down, and the PS-enriched supernatant was dialyzed against distilled water before lyophilization. ^{1}H-NMR spectra were recorded on a Jeol Fx.100 instrument operated in the PFT mode. The spectra were recorded for solutions in D_2O at 85°C with external tetramethylsilane as the internal standard. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was run essentially as described previously (20). The separation gel (142 by 120 mm, 1.5 mm thick) contained 20% acrylamide and 0.45% bisacrylamide. The stacking gel (142 by 40 mm) contained 4.5% acrylamide and 1.2% bisacrylamide. Samples (5 μg per well) were heated for 5 min at 100°C in a sample buffer consisting of a 0.0625 M Tris-hydrochloride buffer (pH 6.8) containing 2% SDS, 10% glycerol, 2% mercaptoethanol, 0.1% Na_2-EDTA, and 0.001% phenol red as the tracking dye. Gels were stained by the silver method (26).

Cell culture media. Standard medium consisted of RPMI 1640 (GIBCO Bio-Cult Ltd., Paisley, Scotland) containing 10% (vol/vol) heat-inactivated (56°C, 30 min) newborn calf serum (GIBCO), 1 mM sodium pyruvate, 100 U of penicillin-streptomycin and 4 mM L-glutamine. For hypoxanthine-aminopterin-thymidine selection standard medium was supplemented with 0.1 mM hypoxanthine (6-hydroxypurin), 0.016 mM thymidine, and 0.4 μM aminopterin (4-aminofolic acid). After 14 days of selection, aminopterin was omitted. A week later, hypoxanthine and thymidine were also excluded.

Standard medium, as described above, without the calf serum was used for serum-free cultures of hybridomas. The medium was supplemented with 1% non-essential amino acids (Flow Laboratories Ltd., Irvine, Scotland), 5 μg of insulin per ml (Sigma Chemical Co., St. Louis, Mo.), and 5 μg of transferrin per ml (Sigma) (3).

Rabbit antisera. Rabbits were immunized twice weekly with increasing doses (0.25 to 2.0 ml) of whole heat-killed bacteria (2×10^9 cells per ml) intravenously. After 3 weeks, blood was drawn and serum was tested. Absorptions of rabbit antisera were performed with whole heat-killed bacteria according to standard manuals (7).

Hybridoma production. BALB/c mice were immunized intraperitoneally on days 0 and 21 with 1×10^8 heat-killed *S. flexneri* bacteria suspended in Freund complete adjuvant and in phosphate-buffered saline (PBS) (pH 7.2), respectively. On day 25, spleen cells from immunized mice were fused with either of the mouse plasmacytoma cell lines FO or Sp2/0, essential-

ly as described by De St. Groth and Scheidegger (4).

At 1 day before fusion, microtiter plates (Titertek; Flow Laboratories) were prepared with 0.2 ml of hypoxanthine-aminopterin-thymidine medium and 5×10^3 mouse peritoneal cells per well. For fusion, spleen cells from three immunized BALB/c mice were washed and a portion (3×10^7 to 1×10^8 cells) fused with 5×10^7 washed myeloma cells in PEG 4000 (E. Merck AG, Darmstadt, West Germany). The cells were spread in 0.05-ml samples in 440 microtiter wells. After 12 to 14 days of growth in selective medium (21) culture supernatants were assayed in enzyme-linked immunosorbent assay (ELISA) against homologous LPS. (Wells with an $A_{405} \times 100$ min of >1.0 were scored as positive.) Subsequently, clones binding to LPS were tested by using seven *S. flexneri* LPS of types 2a, 2b, 3a, 5b, X, Y, and 4bR. Hybridomas giving specific binding to homologous LPS and to LPS carrying homologous antigenic epitopes were recloned twice by limiting dilution or until 100% cloning efficiency was accomplished. Ascites tumors were induced in pristane-treated (2,6,10,14-tetramethylpentadecane; Aldrich Chemical Co., Milwaukee, Wis.) BALB/c mice (23). Approximately 3×10^6 hybridoma cells were administered intraperitoneally. After 7 to 21 days, mice were killed and the ascites fluid was collected.

Purification and characterization of monoclonal antibodies. Ascites fluid was purified by affinity chromatography by using a *Staphylococcus aureus* protein A-Sepharose column (Pharmacia Fine Chemicals, Uppsala, Sweden) (9). Antibody class and subclass were determined by double radial immunodiffusion. Culture supernatants were concentrated 10 times by ammonium sulfate precipitation and tested against rabbit anti-mouse immunoglobulins γ_1, γ_{2a}, γ_{2b}, γ_3, and μ heavy chain antisera and rabbit anti-mouse κ and λ light chain antisera (Litton Bionetics, Kensington, Md.).

Preparation of protein A-containing staphylococci. The preparation of staphylococci was performed essentially as described by Kronvall (19). The bacteria were grown overnight at 37°C in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.). The suspension was washed in PBS at 4°C three times. The bacteria were then treated with 0.5% formaldehyde for 3 h at room temperature. After subsequent washing, the bacteria were suspended to 10% (vol/vol) in PBS and heat treated at 80°C for 10 min. The washings were repeated twice in 0.1 M sodium phosphate buffer (pH 8.0), and the bacteria were finally suspended to 10% as before. The staphylococci were then stored at 4°C until used.

Preparation of sensitized staphylococci. A 0.1-ml amount of ascites was added to 1 ml of the 10% formaldehyde and heat-treated staphylococci suspension. The suspension was kept for 3 h under continuous agitation and then washed twice at 4°C with 0.1 M sodium phosphate buffer, pH 8.0. The sensitized bacteria were resuspended to 2% (vol/vol) in the same buffer and stored at 4°C until used (up to 2 months).

For coagglutination (COA) testing, HgI_2-killed (2 h at 20°C with 0.1% HgI_2, 0.4% KI, 0.04% (vol/vol) formaldehyde, and 0.9% NaCl) *Shigella* strains were boiled for 1 to 2 h and spun, and a small portion was suspended in 0.05 ml of COA reagent on a glass slide, which was rocked back and forth. Agglutination was registered as ++ when clear, as seen by the naked eye within 30 s, and as + if a magnifying glass was needed for observation.

ELISA. ELISA was run in Nunc (A/S Nunc, Roskilde, Denmark) 96-well flat-bottomed microtiter trays (8, 29); 0.1 ml of all LPS (10 μg/ml in 0.05 M carbonate buffer, pH 9.6) was added to each well and kept at 20°C overnight. After coating, 0.1 ml of 1% bovine serum albumin (grade V; Pentex Biochemical, Kankakee, Ill.) in the same carbonate buffer was added, and the trays were kept at 4°C until used. Before use the coated plates were washed three times with 0.15 M NaCl containing 0.05% Tween 20. A 0.1-ml amount of either serum, culture fluids, or ascites (diluted in PBS–0.05% Tween 20) was added, and the plates were incubated for 4 h at ambient temperature (20°C). Trays were washed as before, and an alkaline phosphatase-conjugated rabbit anti-mouse immunoglobulin (diluted in PBS–0.05% Tween 20) purified by immunoadsorption was added (2). Alternatively, a sheep anti-rabbit immunoglobulin was used. (The conjugates have been tested and shown to detect both immunoglobulin G (IgG), IgM, and IgA; unpublished data.) Trays were incubated overnight at room temperature.

For developing, trays were washed as before, and 0.1 ml of a 1 M diethanolamine (J. T. Baker Chemicals B.V., Denventer, Holland) and 0.5 mM $MgCl_2$ (pH 9.8) buffer containing 1 mg of *p*-nitrophenyl phosphate per ml (Sigma) was added. Plates were incubated at 37°C for 100 min and read in a Flow Titertek Multiscan photometer at 405 nm. For endpoint titrations, 10-fold dilutions of 10^{-2} to 10^{-6} in PBS–0.05% Tween 20 were made of the serum or ascites to be tested. After running the test as described, the values for different dilutions were plotted on a logarithmic scale, and the serum dilution giving the absorbance 0.1 at 405 nm × 100 min at 37°C was extrapolated as the endpoint titer.

RESULTS

LPS heterogeneity in *S. flexneri* analyzed by SDS-PAGE. SDS-PAGE of the LPS preparations used in this study revealed a heterogeneity in the amount of O-antigen present in the LPS (Fig. 2). The LPS preparations of serotypes 1b, 3a, 5a, and X had much naked core (without O chains). Thus, there is a possibility that even when using LPS from smooth strains as antigens, antibodies binding to the core region can be selected. This necessitated the use of a chemically characterized LPS from the *S. flexneri* 4bR rough mutant as a negative control (13, 14). Furthermore, all LPS used were assayed by sugar analysis and ^{1}H-NMR and found to be in agreement with published structures (15–17; data not shown).

Specificity and titer of mouse anti-*S. flexneri* type 2a and 2b monoclonal antibodies. Subsequent to two fusion experiments with spleen cells from *S. flexneri* type 2a-immunized mice, 15 clones (1.5% of wells positive for cell growth) with anti-*S. flexneri* type 2a specificity were found; 13 were IgG clones and 2 were IgM clones. None of these reacted with the *S. flex-*

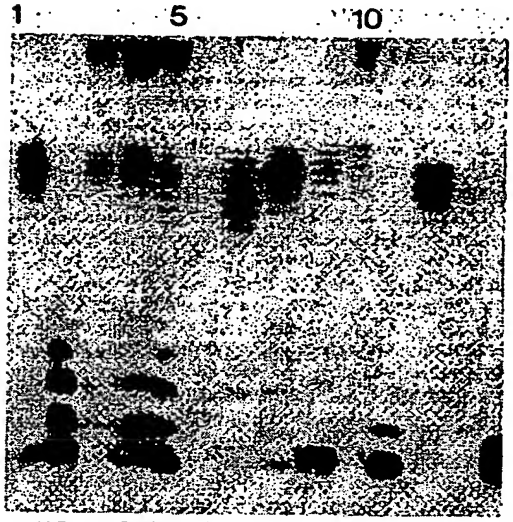

FIG. 2. SDS-PAGE analysis of *S. flexneri* LPS. Samples of 5 μg were subjected to electrophoresis. Slots 1 to 14 show *S. flexneri* serotypes 1a, 1b, 2a, 2b, 3a, 3b, 4a, 4b, 5a, 5b, X, Y, 6, and 4bR (a rough mutant completely lacking O-antigen), respectively.

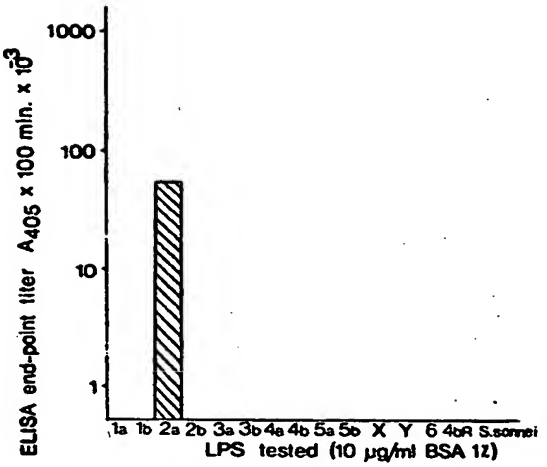

FIG. 3. ELISA endpoint titers of one monoclonal antibody preparation with specificity for one epitope of *S. flexneri* type 2a (II:3,4) [MASF(II:3,4)-1] (IgG_3). Ascites fluid was collected from BALB/c mice, pooled, and assayed.

neri type 2b LPS antigen. Nine clones reacted exclusively with the *S. flexneri* type 2a LPS. The ELISA endpoint titers for one IgG ascites-grown clone [MASF(II:3,4)-1] against the test battery of *S. flexneri* and *S. sonnei* LPS are shown in Fig. 3. All nine IgM and IgG clones showed the same unique specificity for the *S. flexneri* type 2a antigen and no measurable titer against any other tested LPS antigen ($<0.5 \times 10^3$).

Subsequent to one fusion experiment with spleen cells from *S. flexneri* type 2b-immunized mice, 65 clones (15% of wells positive for cell growth) reactive with *S. flexneri* type 2b LPS were found. Of these, 31 were specific for the 7,8 antigenic determinant, 5 reacted exclusively with the type 2b LPS antigen, and 1 reacted with both the type 2a and 2b LPS antigens. The remaining 28 clones showed additional reactivity with non-type II:3,4, II:7,8, or 7,8 antigenic determinants (data not shown). The results of the ELISA titrations, which display excellent specificity for the 7,8 antigenic determinant, for one IgG_3 [MASF(7,8)-1] clone grown as ascites are shown in Fig. 4a, and those for the one IgG_3 clone [MASF(II)-1] showing type 2a and b specificity are shown in Fig. 4b.

Supernatants from serum-free cultures of the clones were concentrated 10-fold by ammonium sulfate precipitation and assayed for subclass specificity by double radial immunodiffusion. Of the clones, 60% had the μ, 33% had the γ_3, and 6% had the γ_1, γ_{2a}, or γ_{2b} heavy chain. Only one clone had the λ light chain, all the rest (98%) had the κ light chain.

Specificity and titer of rabbit anti-*S. flexneri* antisera. Conventional preparation of diagnostic *S. flexneri* antisera involves immunization of rabbits. The ELISA antibody titers against the same test panel of *Shigella* LPS antigens as those used above in serum collected from a rabbit immunized intravenously with heat-killed *S. flexneri* type 2a are shown in Table 1. When several different preparations of absorbed antisera (intended to be specific for the *S. flexneri* II and 7,8 antigenic determinants) used for diagnostic purposes were tested by ELISA they were shown to contain measurable and significant antibody titers against not only the desired antigenic determinant but also against other unwanted antigenic determinants (Table 1).

COA with *S. aureus* Cowan 1 sensitized with the *S. flexneri*-specific monoclonal antibodies. For sensitization of the *S. aureus* Cowan 1, ascites fluid diluted 1:10 and 1:5 was used for the II:3,4 [MASF(II 3,4)-1] and 7,8 [MASF(7,8)-1] clones, respectively. The ascitic fluid titer of the II-specific clone [MASF(II)-1] was too low to sensitize the bacteria. After purification and a fivefold concentration, using affinity chromatography on a protein A-Sepharose column, the antibody preparation was suitable for sensitization.

A total of 211 *S. flexneri* strains were tested in COA with the three monoclonal antibody preparations. All *S. flexneri* type 2a and 2b strains

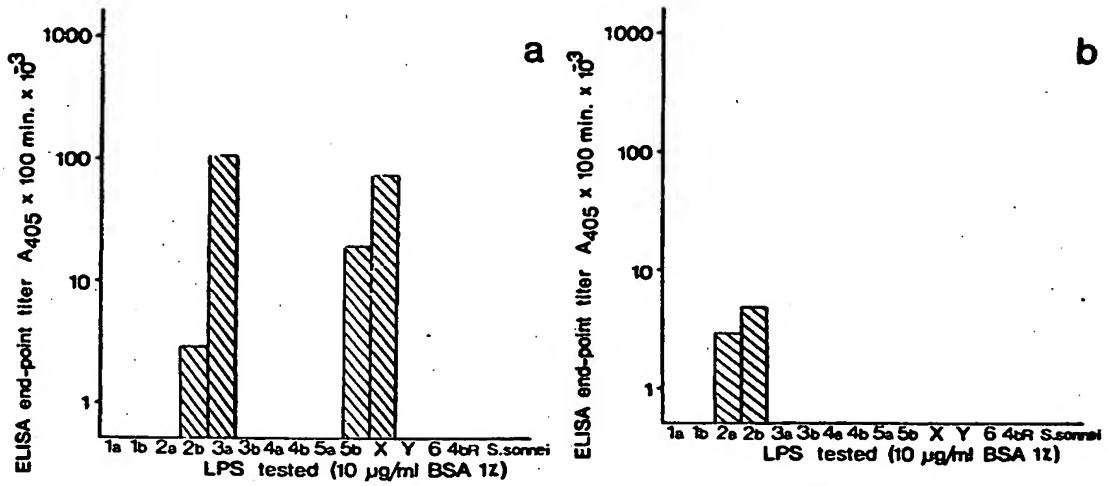

FIG. 4. ELISA endpoint titers of two monoclonal antibody preparations with specificity for different epitopes of *S. flexneri* type 2b (II:7,8). Ascites fluid was collected from BALB/c mice, pooled, and assayed. a, [MASF(7,8)-1] (IgG_3); b, [MASF(II)-1] (IgG_3).

agglutinated the II-specific monoclonal antibody [MASF(II)-1] reagent, whereas no other strains were agglutinated (Table 2). The same excellent specificity was found for the two other antibody preparations; the II:3,4 antibody [MASF(II:3,4)-1] reagent agglutinated only the type 2a strains and the 7,8 antibody [MASF(7,8)-1] reagent agglutinated only the antigen 7,8-containing *S. flexneri* strains.

The clones showing *S. flexneri* type 2b specificity (II:7,8) by ELISA were found unsuitable for COA purposes since they did not show the desired specificity.

Direct agglutination with monoclonal IgM antibodies. Two monoclonal IgM antibody preparations with specificity for either *S. flexneri* type 2a [MASF (II:3,4)-2] or the antigen 7,8 determinant [MASF(7,8)-2] were tested in slide agglutination. Both clones could be diluted, the endpoint titers for agglutination visible with the naked eye were 1:40 for the II:3,4-specific clone, and 1:400 for the 7,8 antigen-specific clone. The specificity was as excellent as that found for the IgG clones (Table 3).

DISCUSSION

The fact that the structure of the O-antigenic PS chain of *S. flexneri* is known (5, 15–17) facilitated the selection and characterization of isolated monoclonal antibodies with specificity for the O-antigen. All LPS used in this study were chemically defined by sugar analysis, ^{1}H-NMR spectroscopy, and in accordance with published data (15–17). In this study, primarily aimed at production of *S. flexneri* serotype 2 monoclonal antibodies, three main groups with specificity for the O-antigen were obtained: (i) antibodies with specificity for serotype 2a and 2b strains [MASF(II)-1] (Fig. 4B, Table 2), (ii) antibodies with specificity for *S. flexneri* type 2a strains only [MASF(II:3,4)-1,-2] (Fig. 3, Table 2, Table 3), and (iii) antibodies with specificity for the 7,8 antigenic determinant, e.g., *S. flexneri* serotypes 2b, 3a, 5b, and X [MASF(7,8)-1,-2] (Fig. 4, Table 2, and Table 3).

It is interesting to speculate to which region of the O-antigenic PS chain these monoclonal antibodies bind. Both classes binding to *S. flexneri* serotype 2 strains apparently interact with the α-D-glucose *p* 1 → 4 α-L-ramnosyl *p*III epitope (Fig. 1). They differ, however, in that the presence of the α-D-glucose *p* 1 → 3 α-D-rhamnosyl *p*I epitope (the 7,8 antigenic determinant as present in serotypes 2b, 3a, 5b, and X) is compatible with binding for the MASF(II) antibodies, but not with binding of the MASF(II:3,4) antibodies (Fig. 3 and 4b, Table 2).

Quantitative inhibition studies with synthetic saccharides identical to various regions of the O-antigenic PS chain will help us define the binding specificity of the antibodies. The conformation of the *S. flexneri* serotype Y O-antigenic PS chain has recently been determined using NMR (1). Such knowledge, when extended to the serotype 2a and 2b PS, will certainly aid our understanding of the molecular basis for the difference in specificity for these two groups of antibodies.

The third group of monoclonal antibodies, MASF(7,8), reacted specifically with the 7,8

TABLE 1. ELISA endpoint titers × 10^{-3} for unabsorbed and absorbed anti-*S. flexneri* rabbit antisera binding to *S. flexneri* LPS[a]

Serum (source)	Endpoint titers for O-antigenic serotype (antigenic formula):											
	1a (I:4)	1b (I:6)	2a (II:3,4)	2b (II:7,8)	3a (III:6,7,8)	3b (III:6,3,4)	4a (IV:3,4)	4b (IV:6)	5a (V:3,4)	5b (V:7,8)	X (−7,8)	Y (−3,4)	6 (VI−)	4bR
Rabbit anti-*S. flexneri* type 2a (NBL)[b]	20	75	26	11	45	85	90	73	28	9	60	75	2	55
Rabbit anti-*S. flexneri* type II (NBL I)[c]	<1	<1	17	6	1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Rabbit anti-*S. flexneri* type II (NBL II)[c]	<1	<1	31	3	3	<1	<1	<1	<1	<1	5	<1	<1	<1
Rabbit anti-*S. flexneri* type X (NBL I)[c]	1	22	3	2	22	9	1	9	1	1	22	<1	<1	<1
Rabbit anti-*S. flexneri* type X (NBL II)[c]	<1	2	<1	9	116	18	5	2	<1	16	31	1	<1	<1
Rabbit anti-*S. flexneri* type X (NBL III)[c]	<1	<1	320	140	1,500	<1	<1	<1	<1	100	310	<1	<1	9

[a] ELISA endpoint titers are the reciprocal serum dilutions giving the absorbance 0.1 at 405 nm × 100 min at 37°C.
[b] Unabsorbed serum.
[c] Type-specific absorbed sera.

TABLE 2. COA using *S. aureus* Cowan 1 sensitized with monoclonal antibodies specific for *S. flexneri*

S. flexneri serotype (antigenic formula)	No. of strains positive by COA/ no. of strains tested for:		
	[MASF (II-)-1] (IgG_3)	[MASF (II:3,4)-1] (IgG_3)	[MASF (7,8)-1] (IgG_3)
1a (I:4)	0/4	0/4	0/4
1b (I:6)	0/17	0/17	0/17
2a (II:3,4)	64/64	64/64	0/64
2b (II:7,8)	27/27	0/27	27/27
3a (III:6,7,8)	0/18	0/18	18/18
3b (III:6,3,4)	0/9	0/9	0/9
4a (IV:3,4)	0/13	0/13	0/13
4b (IV:6)	0/1	0/1	0/1
5a (V:3,4)	0/1	0/1	0/1
5b (V:7,8)	0/7	0/7	7/7
5 non-a, non-b (V:−)	0/11	0/11	0/11
X (−:7,8)	0/29	0/29	29/29
Y (−:3,4)	0/4	0/4	0/4
6 (VI:−)	0/6	0/6	0/6

determinant-carrying strains (Tables 2 and 3, Fig. 4a). This implies that the combining site of these antibodies recognizes the α-D-glucose *p* 1 → 3 α-L-rhamnosyl *p*I region of the O-polysaccharide chain. The ability of these antibodies to bind to all 7,8-carrying isolates (Table 2) indicates that other substituents on the basic Y structure, (Fig. 1) such as the α-D-glucose *p* 1 → 4 α-L-rhamnosyl *p*III in serotype 2b bacteria, the acetyl on O-2 of the α-L-rhamnosyl *p*III residue in serotype 3a bacteria, and the α-D-glucose *p* 1 → 3 α-L-rhamnosyl *p*II in serotype 5b bacteria, do not interfere with the binding to the 7,8 epitope.

The results obtained in the binding studies to the LPS antigens in ELISA were supported to 100% in the COA studies in which a total of 211 clinical isolates of *S. flexneri* were tested (Table 2).

The excellent specificity of the monoclonal antibody preparations was in stark contrast to what was seen when testing the polyclonal rabbit sera (Table 1). It was evident that the unabsorbed rabbit sera had antibody populations which reacted with virtually all of the *S. flexneri* LPS antigens, smooth and rough, i.e., lacking the O chains (Table 1). There are several reasons for this. Firstly, the LPS are not homogeneous, e.g., some of the core chains are not capped by O-antigenic PS chains (11, 24). SDS-PAGE analysis of our *S. flexneri* LPS antigens revealed that in several of them significant amounts of uncapped cores were present (Fig. 2). Secondly, the possibility exists that the D-glucosyl and O-acetyl substituents of the basic tetrasaccharide are not present in stoichiometric amounts. In several instances the substituents are coded for by lysogenizing bacteriophages (10, 12, 22).

TABLE 3. Agglutination with IgM monoclonal antibodies directed against *S. flexneri* O-antigens

S. flexneri serotype	Antigenic formula	No. of positive strains in agglutination/no. of strains tested for:	
		[MASF(II:3,4)-2] (IgM)	[MASF(7,8)-2] (IgM)
2a	II:3,4	10/10	0/10
2b	II:7,8	0/11	11/11
X	−:7,8	0/11	11/11

Such substitutions are often incomplete because of the spontaneous occurrence of nonlysogenic segregants in the bacterial population. Also of importance is the fact that certain structural domains of the *S. flexneri* O-antigenic PS chain most likely will be the same and accessible irrespective of the presence of the known α-D-glucosyl and O-acetyl substituents. This structural microheterogeneity readily explains the rather poor specificity observed also in the absorbed antisera (Table 1). Standard manuals for the preparation of *S. flexneri* diagnostic antisera also emphasize the problems with obtaining monospecific reagents (7).

Diagnostic laboratories may well benefit from the use of these monoclonal reagents, either used for direct agglutination as was done with the IgM clones (Table 3), or when used for sensitizing protein A carrying *S. aureus* as in the COA studies for IgG clones (Table 2).

ACKNOWLEDGMENTS

We thank A. Strömberg for skillful technical assistance.

This study was supported by a grant 80-5589 from the Swedish National Board of Technical Development.

LITERATURE CITED

1. Bock, K., S. Josephson, and D. R. Bundle. 1982. Lipopolysaccharide solution conformation: antigen shape inferred from high revolution ^{1}H and ^{13}C Nuclear Magnetic Resonance Spectroscopy and hardsphere calculations. J. Chem. Soc. Perkin Trans. 2:59–70.
2. Carlsson, H. E., A. A. Lindberg, and S. Hammarström. 1972. Titration of antibodies to *Salmonella* O-antigens by enzyme-linked immunosorbent assay. Infect. Immun. 6:703–708.
3. Chang, T. H., Z. Stepkowski, and H. Koprowski. 1980. Production of monoclonal antibodies in serum free medium. J. Immunol. Methods 39:369–375.
4. De St. Groth, S. F., and D. Scheidegger. 1980. Production of monoclonal antibodies. Strategy and tactics. J. Immunol. Methods 35:1–21.
5. Dimitriev, B. A., Y. A. Knivel, O. K. Sheremet, A. A. Shakhov, N. K. Kochetkov, and I. L. Hofman. 1979. Somatic antigens of *Shigella*. The structure of the specific polysaccharide of *Shigella newcastle* (Sh. flexneri type 6) lipopolysaccharide. Eur. J. Biochem. 98:309–316.
6. Dröge, W., O. Lüderitz, and O. Westphal. 1968. Biochemical studies on lipopolysaccharides of Salmonella mutants. 3. The linkage of the heptose units. Eur. J. Biochem. 4:126–133.
7. Edwards, P. R., and W. H. Ewing. 1972. Identification of enterobacteriaceae, p. 126–131. Burgess Publishing Company, Minneapolis, Minnesota.
8. Engvall, E., and P. Perlman. 1972. Enzyme-linked immunosorbent assay ELISA. III. Quantitation of specific antibodies by enzyme-labeled antiimmunoglobulin in antigen coated tubes. J. Immunol. 109:129–135.
9. Ey, P. L., S. J. Prowse, and C. R. Jenkin. 1978. Isolation of pure IgG_1 IgG_{2a}, IgG_{2b} immunoglobulins from mouse serum using protein A-Sepharose. Immunochemistry 15:429–436.
10. Gemski, P., Jr., D. E. Koeltzow, and S. B. Formal. 1975. Phage conversion of *Shigella flexneri* group antigens. Infect. Immun. 11:685–691.
11. Goldman, R. C., and L. Leive. 1980. Heterogeneity of antigenic-side-chain length in lipopolysaccharide from *Escherichia coli* O111 and *Salmonella typhimurium* LT2. 1980. Eur. J. Biochem. 107:145–153.
12. Itikawa, H. 1964. Immunochemical and biochemical studies on phage-mediated antigenic conversion in *Shigella flexneri*. Jpn. J. Genet. 38:317–327.
13. Jansson, P. E., A. A. Lindberg, B. Lindberg, and R. Wollin. 1981. Structural studies on the hexose region of the core in lipopolysaccharides from enterobacteriaceae. 115:571–577.
14. Johnson, I. H., R. J. Johnson, and D. A. R. Simmons. 1967. The immunochemistry of *Shigella flexneri* O-antigens. The biochemical basis of smooth to rough mutation. Biochem. J. 105:79–87.
15. Kenne, L., B. Lindberg, K. Petersson, E. Katzenellenbogen, and E. Romanowska. 1977. Structural studies of the *Shigella flexneri* variant X type 5a and type 5b O-antigens. Eur. J. Biochem. 76:327–330.
16. Kenne, L., B. Lindberg, K. Pettersson, E. Katzenellenbogen, and E. Romanowska. 1978. Structural studies of *Shigella flexneri* O-antigens. Eur. J. Biochem. 91:279–284.
17. Kenne, L., B. Lindberg, K. Petersson, and E. Romanowska. 1977. Basic structure of the oligosaccharide repeating unit of the *Shigella flexneri* O-antigens. Carbohydr. Res. 56:363–370.
18. Köhler, G., and C. Milstein. 1975. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature (London) 256:495–497.
19. Kronvall, G. 1973. A rapid slide agglutination method for typing pneumococci by means of specific antibody adsorbed to protein A-containing staphylococci. J. Med. Microbiol. 6:187–190.
20. Laemmli, U. K. 1970. Cleavage of structural protein during the assembly of the head of the bacteriophage T4. Nature (London) 227:680–685.
21. Littlefield, J. W. 1964. Selection of hybrids from makings of fibroblasts *in vitro* and their presumed recombinants. Science 145:709–710.
22. Matsui, S. 1958. Antigenic changes in *Shigella flexneri* group by bacteriophage. Jpn. J. Microbiol. 2:153–158.
23. McKearn, T. J. 1980. Method for growing hybridomas in rats or mice, p. 403–404. *In* R. H. Kenneth, T. J. McKearn, and K. B. Bechtol (ed.), Monoclonal antibodies hybridomas: a new dimension in biological analyses. Plenum Publishing Corp., New York.
24. Palva, E. T., and P. H. Mäkelä. 1980. Lipopolysaccharide heterogeneity in *Salmonella typhimurium* analyzed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis. Eur. J. Biochem. 107:137–143.
25. Sawardeker, J. S., J. H. Sloneker, and A. Jeanes. 1965. Quantitative determination of monosaccharides as their alditol acetates by gas liquid chromatography. Anal. Chem. 37:1602–1604.
26. Tsai, C-M., and C. E. Frasch. 1982. A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. Anal. Biochem. 119:115–119.
27. Westphal, O., O. Lüderitz, and F. Bister. 1952. Über die Extraktion von Bakterien mit Phenol/Wasser. Z. Naturforsch. 7:148–155.
28. Voller, A. 1975. Microplate enzyme-linked immunosorbent assay for Chagas disease. Lancet I:426–428.